DISTRIBUTION, CONDITION, AND GROWTH OF NEWLY SETTLED SOUTHERN FLOUNDER (*Paralichthys lethostigma*) IN THE GALVESTON BAY ESTUARY, TX

A Thesis

by

LINDSAY ANN GLASS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2006

Major Subject: Wildlife and Fisheries Sciences

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Chair of Committee,	Jay R. Rooker
Committee Members,	William H. Neill
	Antonietta Quigg
Head of Department,	Delbert M.Gatlin III

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ABSTRACT

Distribution, Condition, and Growth of Newly Settled Southern Flounder (*Paralichthys lethostigma*) in the Galveston Bay Estuary, TX. (May 2006)
Lindsay Ann Glass, B.S., Texas A&M University-Galveston
Chair of Advisory Committee: Dr. Jay R. Rooker

Several flatfish species including southern flounder (*Paralichthys lethostigma*) recruit to estuaries during early life. Therefore, the evaluation of estuarine sites and habitats that serve as nurseries is critical to conservation and management efforts. I used biochemical condition and growth measurements in conjunction with catch-density data to evaluate settlement sites used by southern flounder in the Galveston Bay Estuary (GBE). In 2005, beam-trawl collections were made in three major sections of the GBE (East Bay, West Bay, Galveston Bay), and three sites were sampled in each bay. Within each sampling site, replicate collections were taken from 1) the marsh edge, 2) an intermediate zone, and 3) the open bay. The average size of southern flounder collected was between 12 and 19 mm standard length, and peak densities occurred in January and February. Catch data indicated that numeric densities of southern flounder were significantly greater in East Bay (2.75 per 100 m^2) than in West Bay (0.45 per 100 m^2) or in Galveston Bay (0.91 per 100 m^2). Habitat-specific variation in density was not found. Otolith-based estimates of age indicated that the majority of southern flounder collected were 35-45 days old and derived from early December to early January hatch-dates. Growth rate differences were negligible across bays and habitats, with the average growth rate being 0.40 mm/day (range 0.21-0.76 mm/day). RNA:DNA ratios indicated that newly settled southern flounder in the GBE were in relatively high condition.

Habitat-specific differences in RNA:DNA ratios were not observed; however, ratios were significantly lower in West Bay (average 8.0) than in East Bay (average 9.5) or in Galveston Bay (average 9.8), suggesting the condition of new recruits may vary across the GBE. Findings from this study indicate that southern flounder use a variety of habitats within the GBE during early life, and survival and recruitment success appear favorable regardless of settlement site. As a result, recruitment success of southern flounder may be less a function of the quality of nursery sites/habitats within the GBE than of other factors (e.g., larval supply to the estuary).

ACKNOWLEDGEMENTS

I thank my advisor Dr. Jay Rooker for guidance and support throughout my work. Thanks, Jay, for all the advice, manuscript shaping and editing, and mentoring that have made my Master's degree a valuable learning experience. I also thank Drs. William H. Neill and Antonietta Quigg for serving on my committee and for their reviews and advice on my thesis.

I express sincere gratitude to Dr. Richard Kraus, who kept me going when I wanted to quit. Richard, thanks for challenging me intellectually and pressing me to be a better scientist.

I would like thank all those in the Fisheries Ecology Lab: Jessica Beck, Joe Mikulas, and Yan Cai. Special thanks to Bert Geary and Michelle Zapp for field help, especially on the cold and rainy days, and to the many others who volunteered their time to help me in the field.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Effectively managing estuarine nursery habitats requires an understanding of population dynamics and the causes of variability in recruitment (Houde 1987; Sinclair 1988; Jones 1990; Cushing and Horwood 1994; Leggett and DeBlois 1994; Rooker and Holt 1996). Recruitment of fishes can vary both temporally (several influxes of recruits in a season) and spatially (recruitment to different areas), and recruitment areas can be disjoined from spawning areas (Cowan and Shaw 2002). Usually a spawning ground is fixed, and environmental variation in factors associated with hydrography dictates the number of larvae that are transported to the nursery (Sinclair 1988). Hjort (1914) suggested that food was a limiting factor during the first-feeding stage or "critical period" of larvae, and starvation-induced mortality during this period was an important determinant of recruitment variability. Cushing (1996) expanded Hjort's "critical period" hypothesis and suggested that food-mediated mortality during any part of the larval period would affect larval abundance and recruitment success. Although evidence demonstrating links between food availability and larval abundance is readily available (Leggett and DeBlois 1994; Cowan and Shaw 2002), predation during the larval period is also an important source of mortality and a primary cause of recruitment variability for many species (Houde 1987, 1989).

While mortality during the larval or pre-settlement stage may have large-scale impacts on recruitment of estuarine and marine fishes, post-settlement processes in

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This thesis follows the style of *Estuaries*.

nursery grounds often result in fine-scale adjustments to year class strength (Iles and Beverton 2000). Beck et al. (2001) defined an area as a nursery if a "species occurred at higher densities, avoided predation, and had higher growth rates compared to other habitats." Rapid growth allows juveniles to move out of size-selective predation ranges and into less vulnerable stages (Houde 1987). Nursery grounds help foster rapid growth and improved survival through a combination of factors, the most important being high-quality prey resources, refuge from predators, and suitable physicochemical conditions (e.g. temperature, salinity, dissolved oxygen) (Gibson 1994; Beck et al. 2001). Therefore, the productivity and quality of a nursery habitat is linked to many factors which vary spatially and temporally, and changes in conditions within a nursery may affect year class success (Beck et al. 2001).

Estuaries are vital nursery grounds for several finfish species, including southern flounder (*Paralichthys lethostigma*). Southern flounder are commercially important flatfish throughout the Gulf of Mexico, second to red snapper in price/weight in Texas markets (GSMFC 2000). In addition, southern flounder are sought by recreational fishers, especially during fall spawning runs when adults are especially vulnerable both to bank and boat fishermen. Due to high levels of exploitation over the last few decades, it is not surprising that abundance and total landings of southern flounder have been on the decline since the late 1980s (GSMFC 2000). In 1987 total landings in Texas (commercial and sport fishing) hit a peak at over 500,000 lbs; however, ten years later total landings were down to approximately 150,000 lbs (GSMFC 2000).

Identifying habitats and sites which serve as nurseries to the many flatfish species that recruit to estuaries in early life is critical to conservation and management efforts.

Along the East coast of the United States, several studies indicated that both abiotic and biotic controls of flatfish populations vary over the species range and that collection of more data on temperature, salinity, substrate, food availability and predation in all ranges is needed (Burke et al. 1991; Miller et al. 1991; Burke 1995; Guindon and Miller 1995). Southern flounder have been found to settle on muddy substrate (Burke et. al. 1991), and higher settlement patterns on specific substrates are common among flatfishes (stone flounder, Malloy et al. 1996; yellowfin sole and Alaskan plaice, McConnaughey and Smith 2000; winter flounder, Stoner et al. 2001; and summer flounder, Burke et al. 1991). Apart from substrate, patterns of habitat use for southern flounder and other congeners appear linked to salinity, with densities of post-settlers often higher in low-salinity waters farthest from tidal inlets (Miller et al. 1991; Gibson 1994; Guindon and Miller 1995). Burke et al. (1991) suggested that low salinity waters may offer protection from stenohaline marine predators, as well as diminish numbers of freshwater predators. Drawing conclusions from either sediment type or salinity alone is cautioned because sediment type and salinity are often correlated with temperature and each other, making conclusions based solely on one of these characteristics problematic (Gibson 1994).

The purpose of the present study was to examine large-scale (site-specific) and small-scale (habitat-specific) variation in settlement of southern flounder in the Galveston Bay Estuary (GBE), and to evaluate the recruits' condition and growth jointly so that the value of different bays and habitats to southern flounder could be assessed. Several complementary approaches were used to determine the quality of areas occupied by newly settled southern flounder along the upper coast of Texas. Specifically, biochemical condition (RNA:DNA) and growth (otolith microstructure analysis) were

used to measure the relative fitness of individuals because poor nutritional condition and slow growth are coupled to survival. Thus, these two measures were used in conjunction with density data to evaluate the relative value of areas occupied by post-settlement southern flounder. Two hypotheses were tested: $H_{0,1}$ Post-settlement southern flounder settle non-selectively throughout the Galveston Bay Estuary (GBE), utilizing all bays and habitats within this complex to the same extent; $H_{0,2}$ Condition and growth (proxy for nursery habitat quality) of post-settlement southern flounder does not vary significantly among bays or habitats within the GBE.

Specific objectives of this study:

1) Describe the general early-juvenile flatfish assemblage in the GBE;
2) Characterize settlement patterns of southern flounder at large scales (across bays) and small scales (across habitats within a bay) in the GBE;
3) Determine the age, hatch-date distribution, and growth of post-settlement southern flounder in different bays and habitats within the GBE; and
4) Establish the habitat quality of different areas inhabited by post-settlement southern flounder in the GBE using measures of biochemical condition and recent growth.

METHODS

Field Work

The GBE was separated into three main areas for study: East Bay, Galveston Bay, and West Bay (Fig. 1). East Bay is the eastern portion of the estuary and is connected to the Gulf of Mexico at Rollover Pass. Galveston Bay consists of sites in the central, core portion of the estuary, with water flow through Bolivar Roads (main entrance to estuary and shipping lanes). West Bay is located in the far western portion of GBE, with its main input of water from San Luis Pass. In each bay, three sites were randomly chosen for repeated sampling: East Bay (site 1: 29°26.96N, 94°41.85W; site 2: 29°29.14N, 94°36.23W; site 3: 29°31.86N, 94°33.72W), Galveston Bay (site 1: 29°20.70N, 94°49.37W; site 2: 29°18.24N, 94°56.68W; site 3: 29°15.30N, 94°55.15W), West Bay (site 1: 29°02.73N, 95°10.51W; site 2: 29°02.04N, 95°11.71W; site 3: 29°03.87N, 95°10.03W). Collections were taken monthly during the primary recruitment period for southern flounder (January to May 2005). Data from preliminary sampling in 2004 were not included in this analysis due to low numbers collected of newly settled southern flounder (n =30; Appendix 1). Each site (9 total) was sampled with a 1 m (W) x 0.5 m (H) beam trawl fitted with a 1mm mesh net attached. Three habitat types were sampled in replicates of three: 1) marsh edge, 2) intermediate zone (10-20m from marsh interface; ~1m depth), and 3) bay zone (depth >1m; typically >100m from marsh interface). In marsh edge and intermediate zones, the net was fitted with two 20-m ropes, researchers walked in an arc away from the path of the net, and then pulled the net to themselves by hand. In the bay zone, the net was attached to a bridle rope (10 m), which

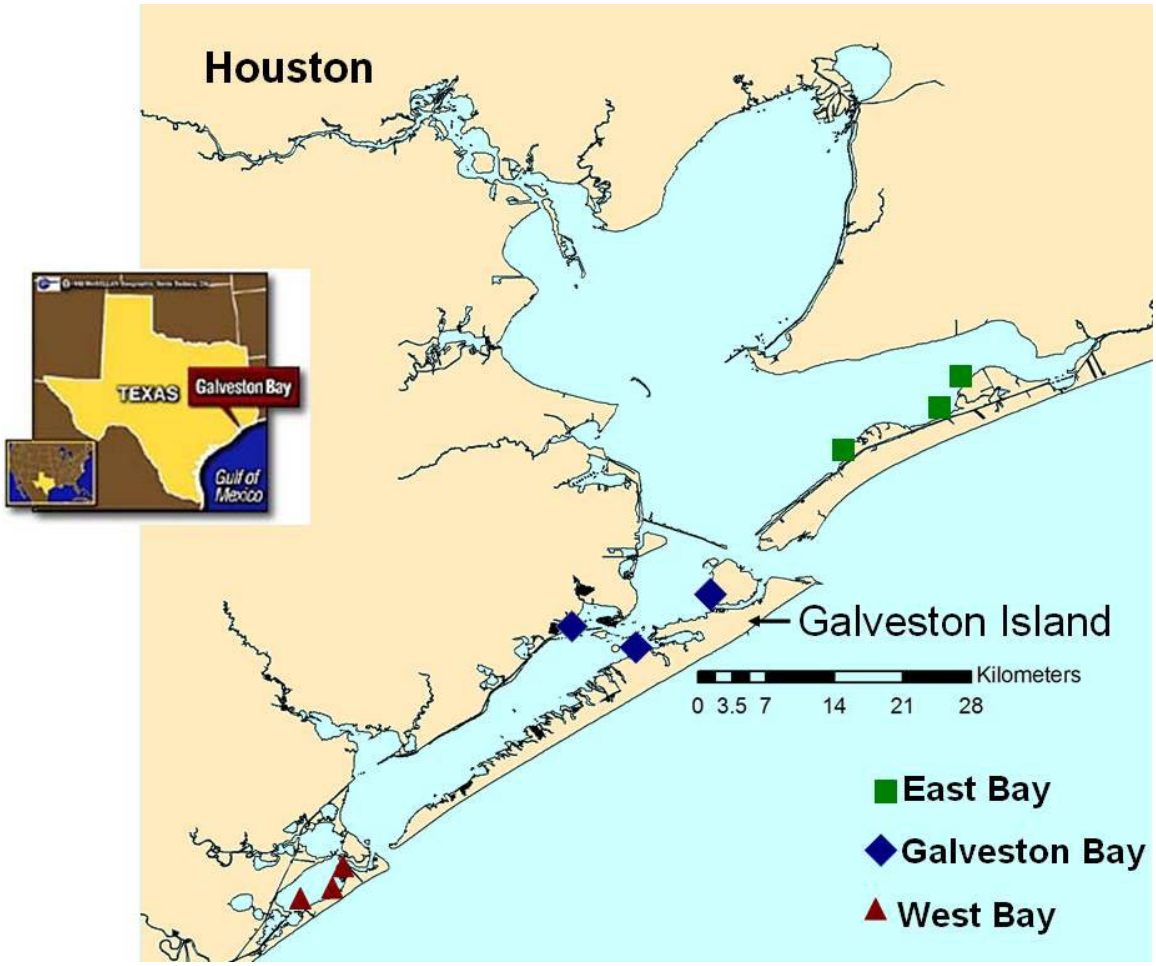

Figure 1. Map of study area and sampling sites in the Galveston Bay Estuary.

was then attached to the back of a boat. The net was pulled at approximately 3.5 km h^{-1} for 2 min, and start and stop GPS locations were recorded for each tow to determine exact location and distance covered. Measurements of temperature, salinity, and DO were taken at each site in the field. These sampling activities occurred between the hours of 9 AM and 5 PM.

Laboratory Work

Condition

Condition of juvenile fish can be determined by a variety of biochemical means by quantifying components of energy substrates or calculating physiological rate indicators, such as proteins, lipids, digestive enzymes, and nucleic acids (Ferron and Leggett 1994). The relationship between the constant quantity of DNA in a cell and the quantity of RNA, which fluctuates with protein synthesis rate, can be linked to growth since growth is achieved through protein synthesis (Buckley et al. 1984; Ferron and Leggett 1994). RNA:DNA ratios reflect recent growth conditions (2-4 days preceding capture) and thus can be connected to environmental measures taken concurrent with sampling (Buckley 1984; Bulow 1987; Ferron and Leggett 1994). This approach has been used successfully to characterize the nutritional status of estuarine fishes (Westerman and Holt 1988; Imsland et al. 2002) including flounder (Gwak and Tanaka 2002).

In the present study, sections of trunk muscle tissue were extracted from frozen juvenile southern flounder for RNA:DNA analysis. Only a subset of southern flounder were analyzed from one sampling period (month: February) to reduce potential effects of temporal variation. Analysis was conducted at the University of Texas Fisheries and

Mariculture Laboratory at Port Aransas, TX, following the ethidium bromide flourometric procedures described by Westerman and Holt (1988). Individual trunk muscle samples were homogenized, and aliquots of homogenates (25-100µl) were used to estimate DNA and RNA concentrations. Calculations were based on comparisons with DNA-EB and RNA-EB calibration curves from known standards. Calf thymus DNA and yeast RNA (type III) were used as standards. Also, aliquots were used for total soluble protein concentration using Bicinchoninic Acid Protein Assay Kit (Sigma product).

Age and Growth

Examination of otoliths is a technique to determine age and long-term growth rates of individuals (Campana and Neilson 1985). Annual layers, or annuli, are formed at a constant, consistent frequency and can be counted for age in years, and distances between successive annuli can be interpreted as being proportional to changes in body size (Campana and Neilson 1985). Also, the otoliths of young fishes form daily checks, or circuli (Pannella 1971). Fitzhugh and Rice (1995) determined that southern flounder during the first year of life produce daily otolith growth increments that can be seen under a microscope. Age and growth of southern flounder based on otolith structure has been used in several studies (Fitzhugh et al. 1996; Stunz et al. 2000; Fischer and Thompson 2004).

Upon the completion of standard length measurements of southern flounder, sagittal otoliths were removed and cleaned with one of the pair randomly selected for aging. The selected otolith was mounted on a slide and polished to the core on each side using Buehler Carbimet paper discs (320, 400, 600, 800, and 1200 grit) and 0.3µm alumina polishing compound following procedure outlined by McCurdy et al. (2002).

Polished sections were examined using an Olympus BX41 compound microscope with analysis aided by Image Pro Plus 4.5 software. Daily growth increments of all otoliths were counted by one reader with a subset counted by a second reader for quality control. Otolith counts that were not within 10% agreement with one another were counted a third time to try to resolve the difference; if it was not resolved, then it was not included for analysis. Otoliths that were considered unreadable (due to cracking, polishing error, or position in epoxy) and otoliths ruined by epoxy drying were also discarded. Of all southern flounder collected, 54% (n = 101) were assigned age, 24% were deemed unreadable due to polishing error (including cracking that might have occurred during polishing), 14% were unreadable or lost due to epoxy problems, and 10% were deemed unuseable due to structure of otolith or count disagreement. Growth rates (daily instantaneous) were determined by fitting an exponential model to size-at-age plots:

$$L_t = L_0 e^{gt}$$

where L_t= length (mm standard length) at time t, L_0 = estimated length at hatching, g = instantaneous growth coefficient, and t = estimated age (days after hatching). Also, growth increment widths between days 30 to 40 were measured to estimate recent growth in a subset of individuals. The most recent 10 increments were not counted because clarity around the edges of the otoliths was poor. Hatchery-reared southern flounder of known age from The University of Texas Marine Science Institute were used to validate daily increment deposition.

Data Analysis

Analysis of variance (ANOVA) tested the main effects of area, site, and habitat type on the density of southern flounder. Data from April were not included in the

analysis because only one southern flounder was caught during this period. Date was used as a blocking factor. Instantaneous growth rates of juvenile southern flounder were estimated as slope of standard length regressed on age estimated from otolith analysis. Analysis of covariance (ANCOVA) was used to investigate effects of bay and habitat on RNA:DNA ratios and growth. The covariate in all ANCOVA models was standard length. Alpha level was set at 0.05.

RESULTS

Environmental Conditions

Salinity varied both spatially and temporally within the GBE. Mesohaline conditions typically were observed in East Bay (mean range: 9-13 ppt), but conditions within the central portion of Galveston Bay (15-25 ppt) and West Bay (20-31 ppt) were essentially polyhaline (Table 1). Significant differences in salinity occurred among bays (ANOVA, $p < 0.001$) and among months (ANOVA, $p < 0.001$) with a bay*month interaction (ANOVA, $p<0.001$); however, salinity did not vary among habitat types (ANOVA, $p = 0.735$). Tukey HSD test indicated that East Bay had significantly lower salinity than both Galveston Bay and West Bay and that all bays were significantly different from one another. Temperature also varied significantly among bays (ANOVA, $p = 0.003$) and among months (ANOVA, $p < 0.001$) with the bay*month interaction included in the model (ANOVA, $p < 0.001$); but, temperature did not vary as a function of habitat type (ANOVA, $p = 0.632$). Tukey HSD test indicated that East Bay (mean 16.8°C) had significantly lower temperatures than both Galveston Bay (mean 17.0°C) and West Bay (mean 18.5°C), with no difference in temperature between Galveston Bay and West Bay. Dissolved oxygen (DO) levels (during the diurnal period) were not significantly different among bays (ANOVA, $p = 0.582$) but were significantly different among months (ANOVA, $p = 0.003$) with no bay*month interaction (ANOVA, $p = 0.061$). Tukey HSD test indicated that January (9.94 mg/mL) and February (9.41mg/mL) had higher DO levels than April (7.82 mg/mL). In contrast to salinity and temperature, DO varied among habitat types in the GBE (ANOVA, $p < 0.001$); higher DO concentrations were found in the marsh edge and intermediate zones than in the bay zone.

Table 1. Environmental parameters (salinity, temperature, and dissolved oxygen) from three regions of Galveston Bay Estuary in 2005. Estimates are mean value (± 1 SE) of the three sites surveyed within each bay. Salinity is reported in ppt, temperature in °C, and dissolved oxygen in mg/L.

Salinity ppt	**January**	**February**	**March**	**April**
East Bay	12 (±0.61)	13 (±0.76)	9 (±0.0.40)	13 (±1.42)
Galveston Bay	19 (±0.68)	16 (±1.63)	15 (±1.21)	25 (±1.29)
West Bay	25 (±0.58)	22 (±0.33)	20 (±0.50)	31 (±0.40)
Temperature °C	**January**	**February**	**March**	**April**
East Bay	13.4 (±0.99)	15.1 (±0.50)	16.8 (±1.65)	21.9 (±0.78)
Galveston Bay	17.1 (±0.92)	18.1 (±0.53)	16.6 (±0.84)	22.7 (±0.42)
West Bay	18.9 (±0.66)	13.1 (±0.22)	18.3 (±0.36)	23.7 (±0.28)
Dissolved Oxygen mg/mL	**January**	**February**	**March**	**April**
East Bay	10.02 (±0.25)	8.95 (±0.67)	7.97 (±0.47)	7.57 (±0.46)
Galveston Bay	10.43 (±0.58)	9.92 (±2.29)	9.78 (±0.45)	7.87 (±0.54)
West Bay	9.93 (±0.44)	9.53 (±1.06)	8.88 (±0.58)	8.03 (±0.66)

Distribution and Abundance

Spatial variation in the assemblage of larval and early-juvenile flatfish present in GBE in 2005 was investigated. A total of 573 individuals was collected from four different species: 335 bay whiff (*Citharichthys spilopterus*), 184 southern flounder (*Paralichthys lethostigma*), 50 blackcheek tonguefish (*Symphurus plagiusa*), and 4 fringed flounder (*Etropus crossotus*) (Fig. 2). In East Bay, southern flounder made up 54% of the overall catch, with bay whiff making up 31% of the catch. In Galveston Bay, bay whiff was the dominant flatfish accounting for 65% of the catch, compared to 28% for southern flounder. Bay whiff also dominated by number and percentage (90%) in West Bay, with southern flounder making up only 7% of the catch. Most southern flounder (82%) collected in GBE were caught in January and February, while most bay whiff (68%) were caught in March and April. Fringed flounder were only caught in the month of February and made up <1% of the flatfish assemblages in the three bays. Blackcheek tonguefish were caught primarily in East Bay (82%), and over 70% were caught in January and February.

Density of southern flounder recruits in GBE varied by time and location, and settlement began at approximately 9 mm (Fig. 3). The catch was dominated by individuals between 12 and 19 mm, with the majority of southern flounder collected from East Bay (77%). Densities of southern flounder ranged from zero in some habitats and months to a maximum of 21.7 per 100 m^2. Significantly higher densities of southern flounder were found in East Bay relative to West Bay and Galveston Bay (ANOVA, $p = 0.006$) (Fig. 4). Sample numbers in East Bay were sufficient to enable

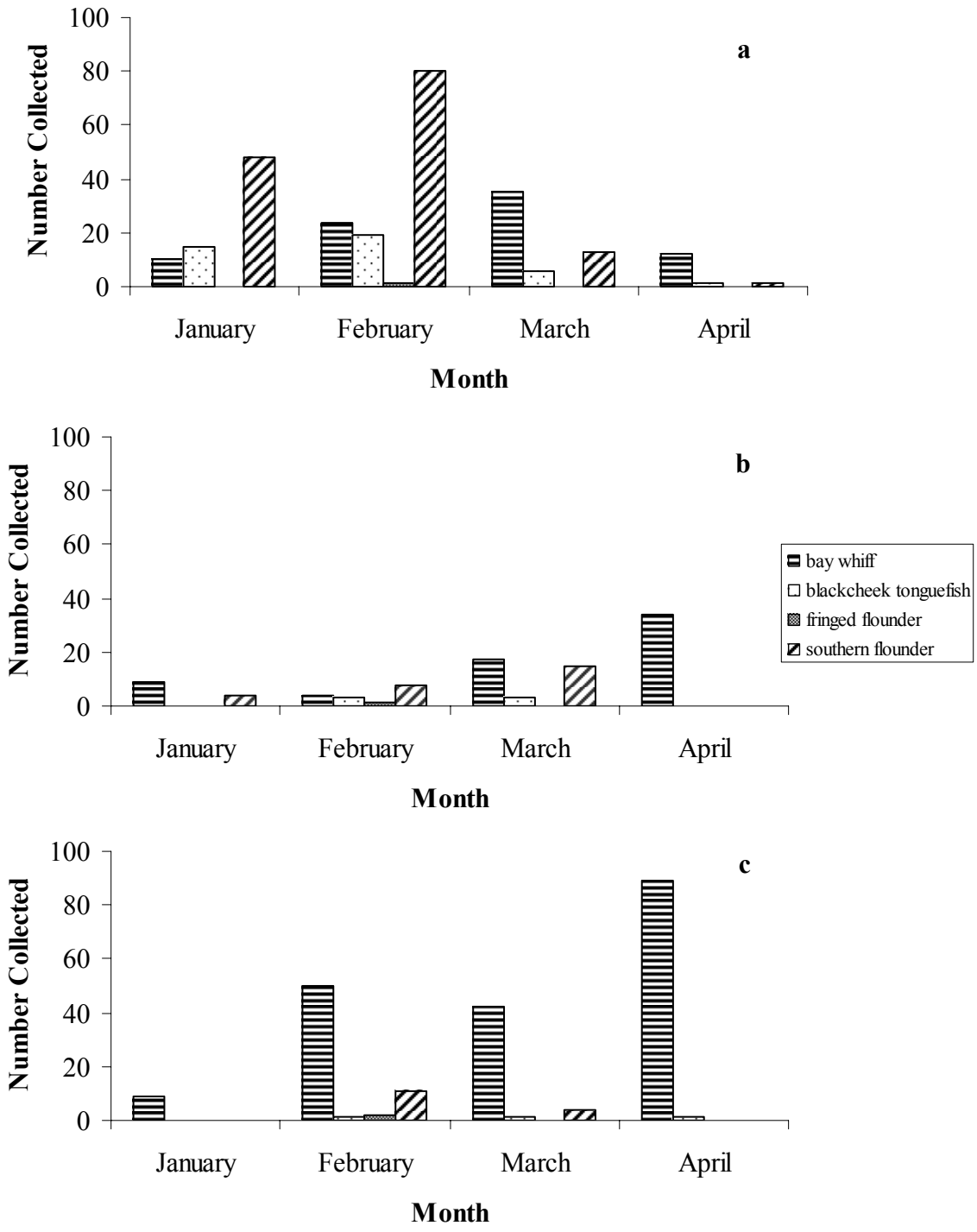

Figure 2. Seasonal variation in the number of larval or juvenile flatfishes (bay whiff, blackcheek tonguefish, fringed flounder, and southern flounder) from the Galveston Bay Estuary in 2005. Data are divided by bay: a) East Bay, b) Galveston Bay, and c) West Bay.

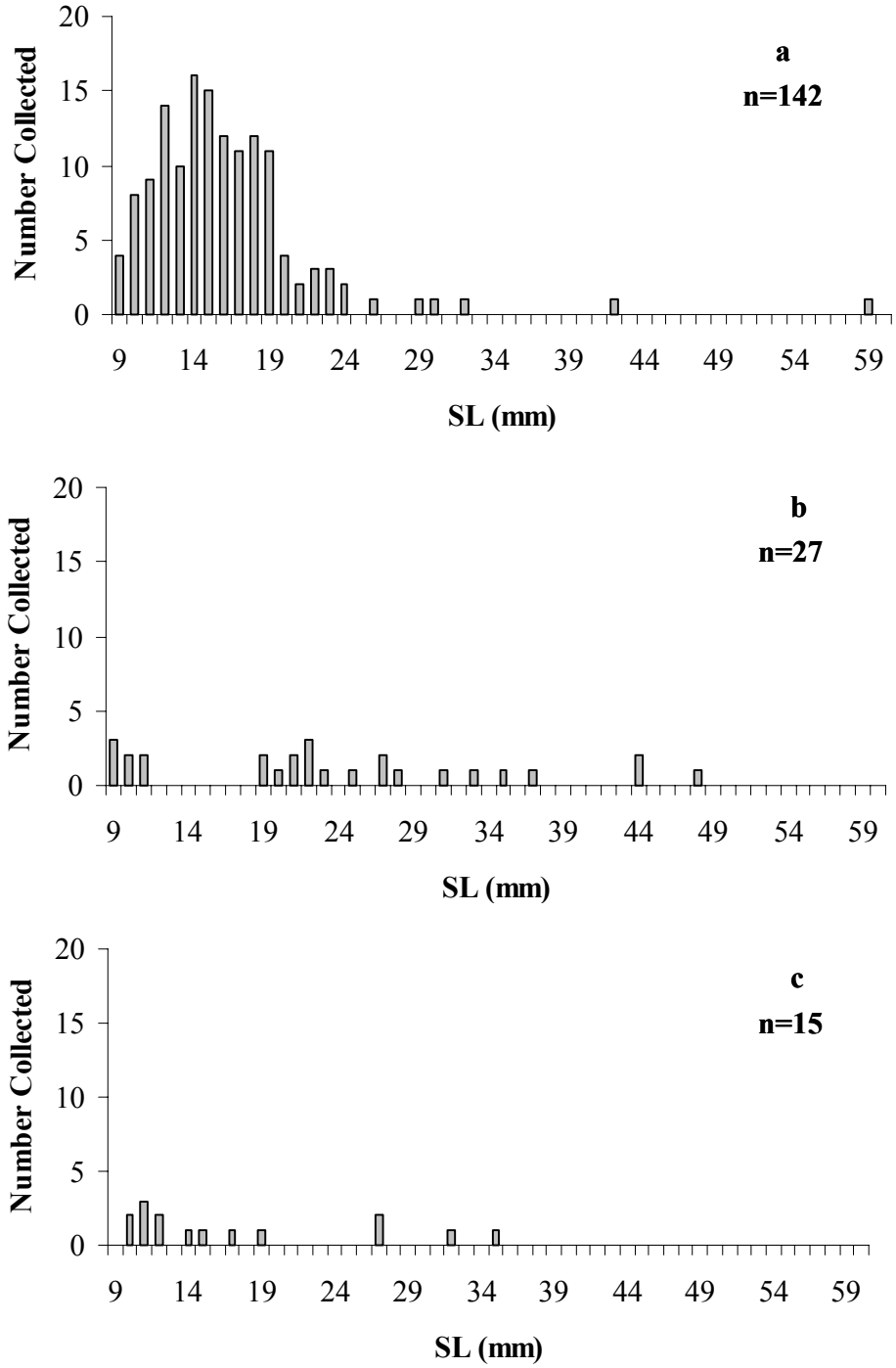

Figure 3. Length-frequency distributions of newly settled southern flounder from the Galveston Bay Estuary in 2005. Data are divided by bay: a) East Bay, b) Galveston Bay, and c) West Bay.

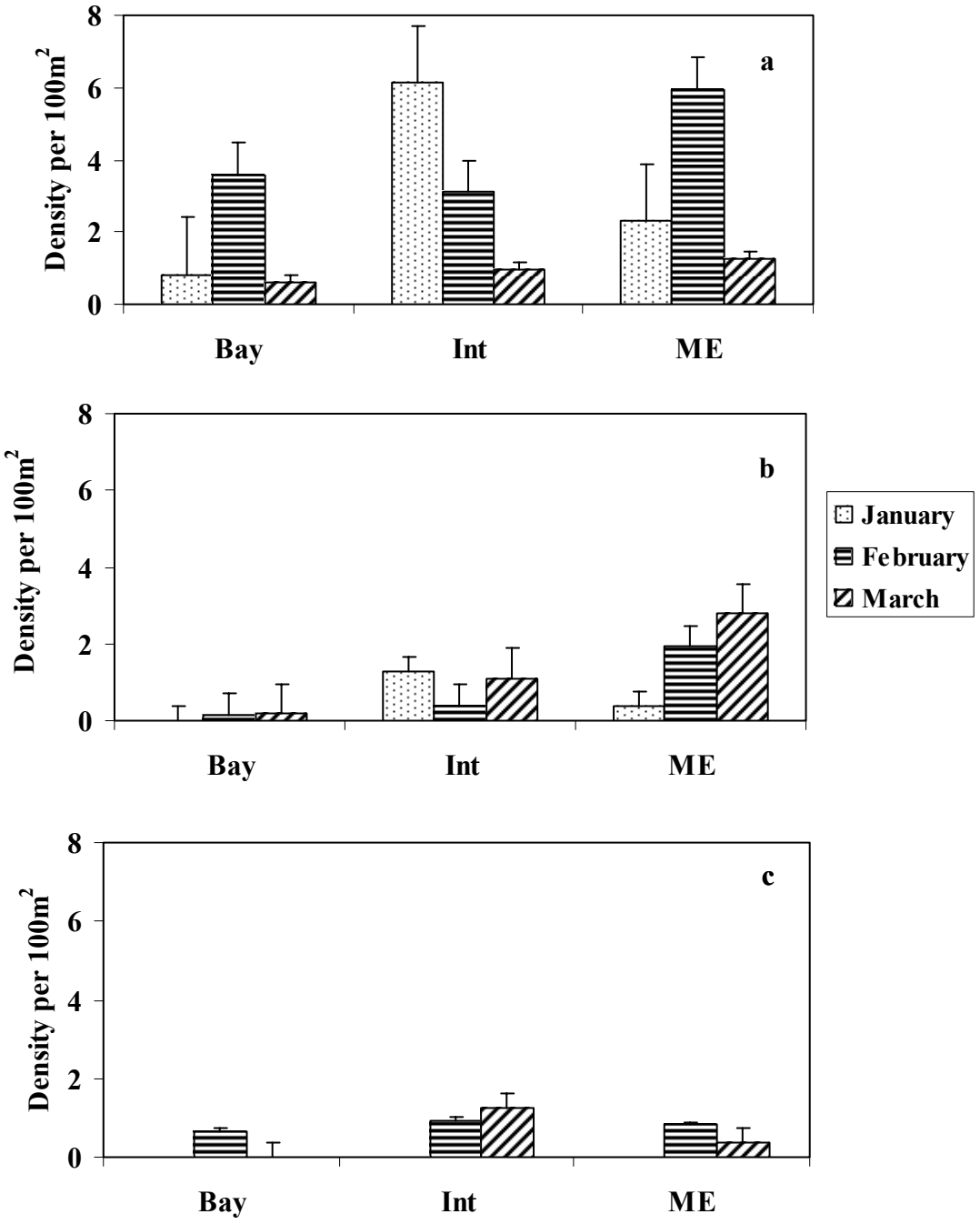

Figure 4. Densities (number per 100m^2) of newly settled southern flounder from the Galveston Bay Estuary in 2005. Estimates of density are given by habitat and data are divided by bay: a) East Bay, b) Galveston Bay, and c) West Bay. Error bars represent 1 SE. Habitat codes: Bay = bay zone, Int = intermediate zone, ME = marsh edge.

investigation of specific patterns of habitat use. Southern flounder densities in the bay-zone habitat (1.7 per 100 m^2) tended to be lower than in the intermediate (3.4 per 100 m^2) or marsh-edge (3.2 per 100 m^2) habitats; however, no significant difference was detected (ANOVA, $p = 0.404$). Month collected was not significant (ANOVA, $p = 0.065$), but densities tended to be higher in January (1.2 per 100 m^2) and February (1.9 per 100 m^2) than in March (0.95 per 100 m^2).

Condition

Estimates of biochemical condition were limited to samples from the same month of collection (February), to minimize temporal effects (e.g., changes in temperature and associated variables). The average RNA:DNA ratio was 9.17 (range: 6.34 to 15.19), and RNA:DNA ratio did not vary with standard length of southern flounder (ANCOVA, $p = 0.52$) (see Appendix 1). Based on a previous laboratory validation study that quantified RNA:DNA ratios of well-fed and starved southern flounder reared at a constant temperature of 18°C and salinity of 31 ppt (G. Joan Holt, unpublished data), over 90% of the assayed southern flounder from the GBE matched the "well fed" signature (Fig. 5). A significant difference in RNA:DNA ratios was detected among bays (ANOVA, $p = 0.003$). West Bay had lower RNA:DNA ratios (mean 8.0 SE ± 0.3) than East Bay (mean 9.5 SE ± 0.2) and Galveston Bay (mean 9.8 SE ± 0.4) (Fig. 6). Tukey HSD test showed that West Bay had significantly lower ratios than both East Bay and Galveston Bay, but there was no difference between East Bay and Galveston Bay. RNA:DNA ratios did not vary by habitat within bay (ANOVA, $p = 0.114$) and no interaction was detected for bay*habitat (ANOVA, $p = 0.632$).

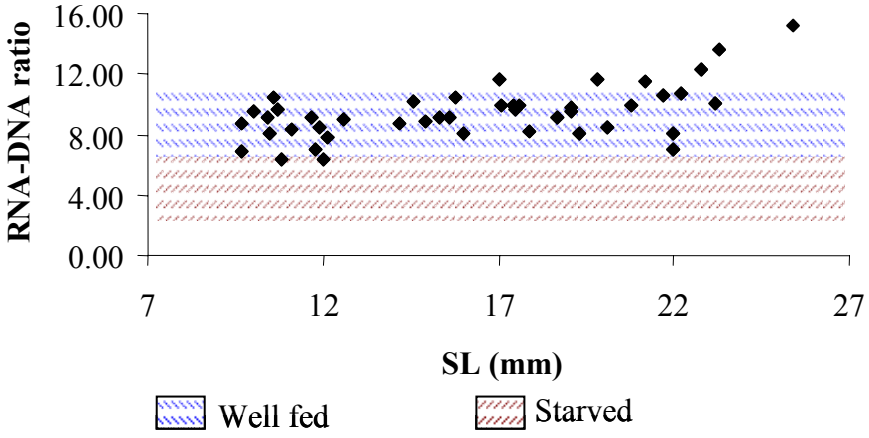

Figure 5. RNA:DNA ratios of newly settled southern flounder from the Galveston Bay Estuary in 2005. Estimates are shown in relation to predicted conditions for well-fed and starved southern flounder from laboratory trials (G. Joan Holt, unpublished data).

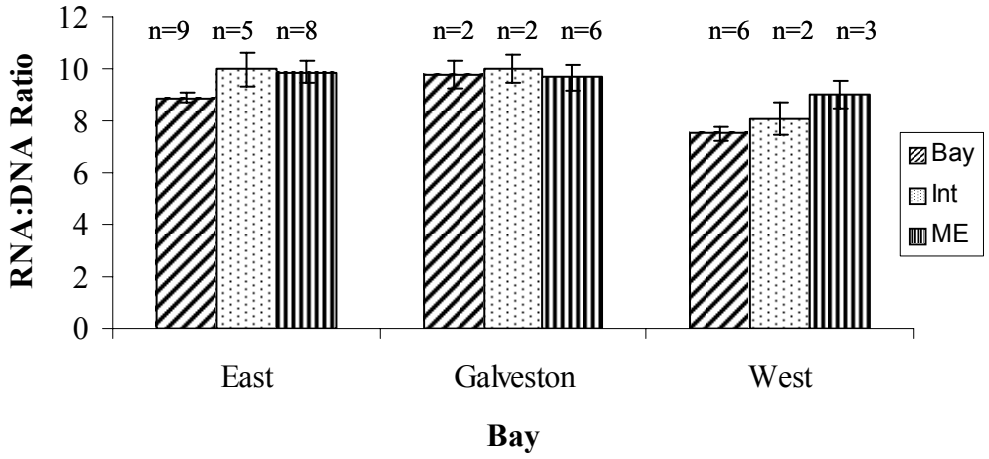

Figure 6. Mean RNA:DNA ratios for newly settled southern flounder from the Galveston Bay Estuary in 2005. Ratios are given by bay and habitat. Error bars represent 1 SE. Habitat codes: Bay = bay zone, Int = intermediate zone, ME = marsh edge.

Age and Growth

Otolith-based estimates of age indicated that southern flounder collected in GBE ranged in age from 27 days old to 78 days old (Fig. 7). Otoliths from 101 individuals were aged; however, for the growth-curve analysis only 100 individuals were used, because length data on one individual had been lost. Over half of the southern flounder aged (53%) were between 35-45 days old. Recruitment of southern flounder to the GBE occurred in a single mode, with a peak in hatch-dates from December 9 thru January 12 (Fig. 8). Hatch-date distributions of individuals collected in East Bay and Galveston Bay were protracted (early December to February), while recruits from West Bay were derived from a limited hatch-date distribution (December 31 to January 11).

Overall growth of southern flounder (all bays combined) was adequately described by the exponential model: SL (mm) $= 5.519e^{0.0253*\text{age-in-days}}$ with an $R^2 = 0.581$ (Fig. 9). Growth rates were 0.24 mm d^{-1} (21-30 days old), 0.31 mm d^{-1} (31-40 days old), 0.40 mm d^{-1} (41-50 days old), and 0.51 mm d^{-1} (51-60 days old), with an average growth rate of 0.40 mm d^{-1}. Although sample sizes were small, habitat- (within East Bay) and bay-specific growth rates were examined; no effect was observed for either habitat (ANCOVA, $p = 0.436$, power = 0.189) or bay (ANCOVA, $p = 0.514$, power = 0.160) (Fig. 10). Southern flounder were divided into cohorts by hatch-date (December and January) and no significant differences were present between cohorts (ANCOVA, $p = 0.172$, power = 0.276) (Fig. 11).

In addition to comparing overall growth for the entire pre-settlement/post-settlement period, recent growth estimates were obtained by measuring widths of outer growth increments (corresponds to growth from 30-40 days) on a subset of otoliths

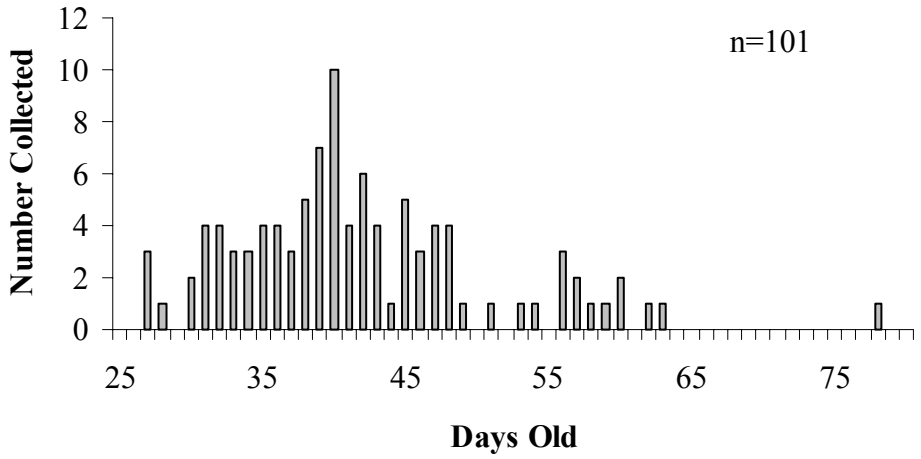

Figure 7. Age-frequency distribution of newly settled southern flounder from the Galveston Bay Estuary in 2005.

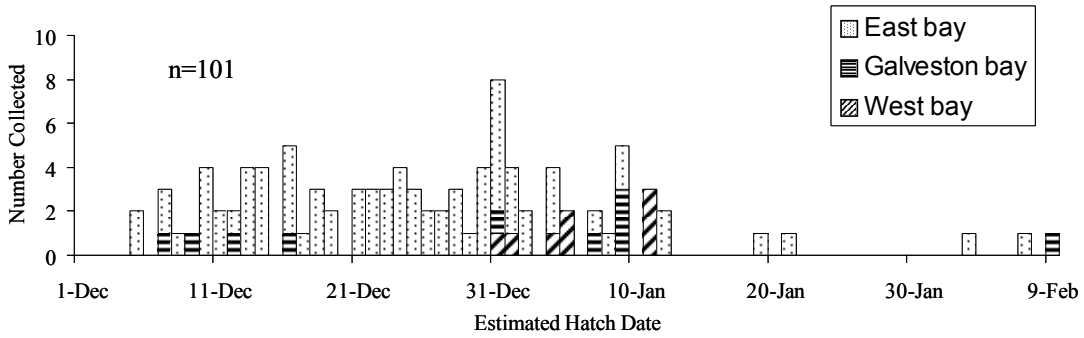

Figure 8. Hatch-date distribution of newly settled southern flounder from the Galveston Bay Estuary in 2005. Data are coded by bay.

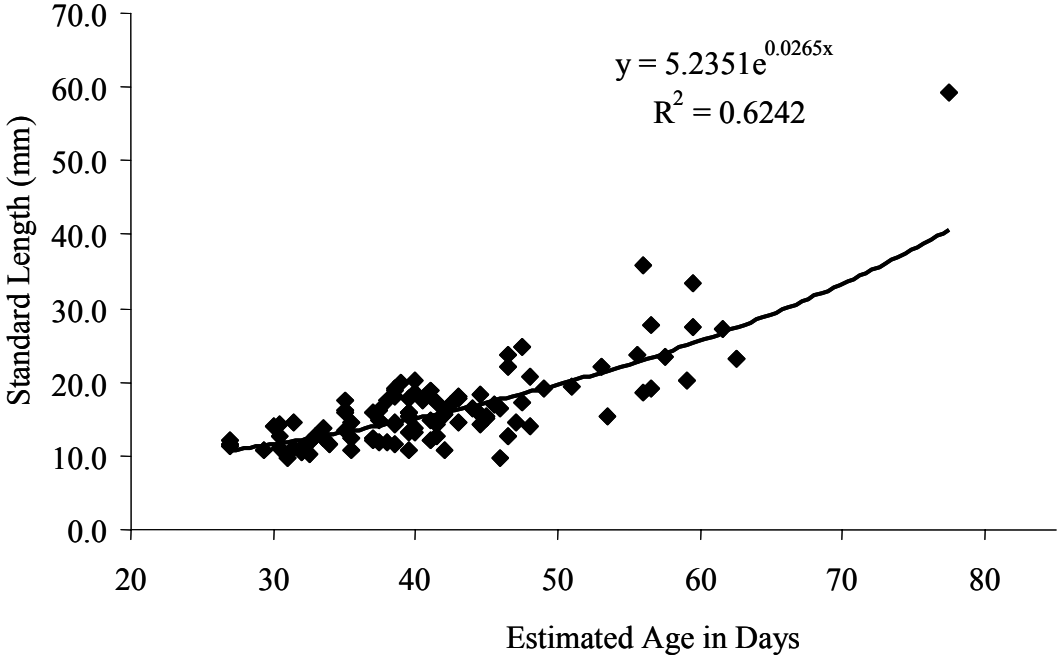

Figure 9. Size-at-age relationship for newly settled southern flounder from the Galveston Bay Estuary (all sites pooled) in 2005 (n = 100). Exponential growth equation is given.

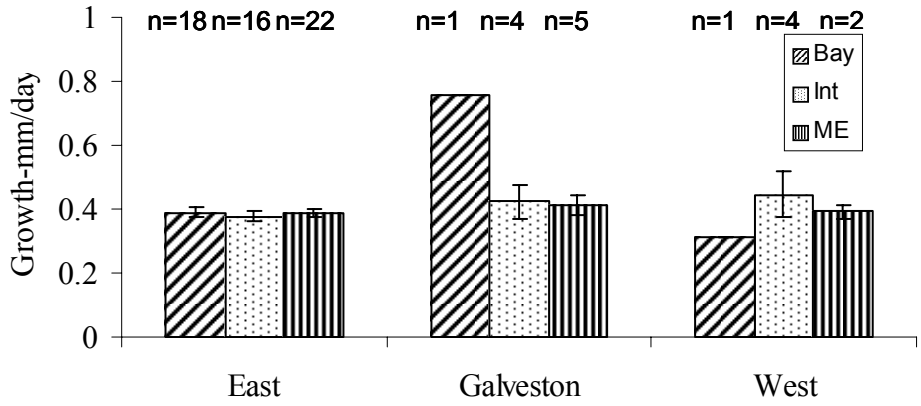

Figure 10. Mean growth rates (mm/day) of newly settled southern flounder from the Galveston Bay Estuary in 2005. Estimates are given by bay and by habitat. Error bars represent 1 SE. Habitat codes: Bay = bay zone, Int = intermediate zone, ME = marsh edge.

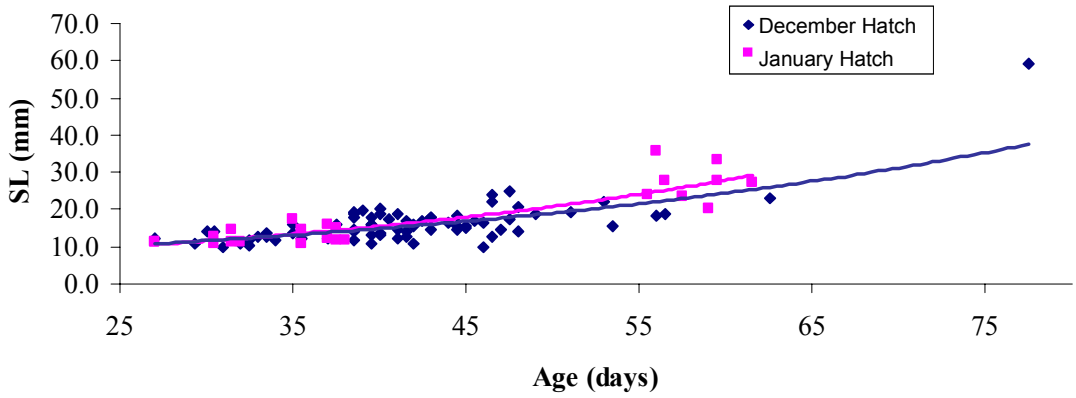

Figure 11. Growth rates of newly settled southern flounder collected from the Galveston Bay Estuary in 2005, separated into hatch-date cohorts (December and January).

representing each bay (all habitats pooled for each bay). Mean estimates of recent otolith growth were highest for southern flounder from West Bay (59.6 microns) and slightly lower in Galveston Bay (53.8 microns). The lowest estimates of recent growth were observed in East Bay (44.1 microns). Despite the trend, no significant difference in recent growth was detected among bays (ANOVA, $p = 0.145$, power = 0.383) or among habitats; nor, did SL vary significantly (ANCOVA, $p = 0.392$) (Fig. 12).

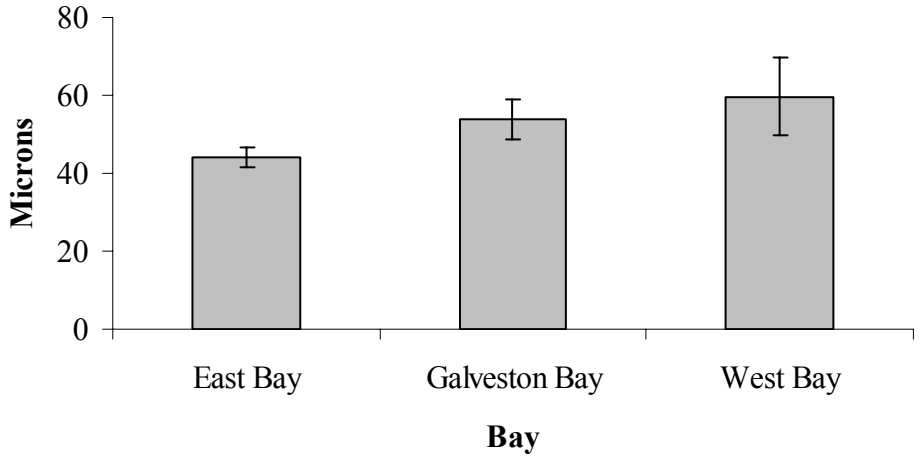

Figure 12. Recent growth of newly settled southern flounder from the Galveston Bay Estuary in 2005. Estimates represent the mean otolith width (microns) from increment 30 to 40. Mean values are given by bay and error bars represent 1 SE.

DISCUSSION

Bay whiff and southern flounder were the dominant juvenile flatfishes collected in the Galveston Bay Estuary (GBE) during the winter 2005 sampling period (58% and 32% of total catch, respectively). In addition, small numbers of juvenile blackcheek tonguefish and fringed flounder were present. The observed flatfish assemblage was consistent with expectations based on presumed winter spawning periods of flatfishes in the Gulf of Mexico (Hoese and Moore 1998; McEachran and Fechhelm 2006); however, comparisons with other estuaries suggest that some regional differences occur. Allen and Baltz (1997) observed all four species in a nearby Louisiana estuary and reported that offshore tonguefish (*Symphurus civitatium*) and bay whiff comprised almost 80% of the flatfish catch, with lower numbers of southern flounder, blackcheek tonguefish, and fringed flounder. In addition, several other species of flatfish were collected including lined sole (*Achirus lineatus*) and hogchoker (*Trinectes maculates*). Taxa observed in both Texas and Louisiana estuaries also occur in the northeastern Gulf, where increased catches of juvenile Gulf flounder (*Paralichthys albigutta*) have been reported (Ginsburg 1952; Topp and Hoff 1972; Nall 1979). Although no juvenile Gulf flounder were collected in the present study, they do occur in the region, albeit in lower numbers than in Florida (Hoese and Moore 1998).

On the East coast of the U.S., all four flatfish taxa collected in the GBE have been observed but their relative abundances are markedly different. For example, Reichart and van der Veer (1991) reported catches of juvenile flatfishes from a Georgia estuary with fringed flounder dominating the catch numerically. High densities of bay whiff and

blackcheek tonguefish were also present. Although southern flounder and two congeners were collected (summer flounder *Paralichthys dentatus*, and fourspot flounder *Paralichthys oblongus*), numbers were low (southern flounder <0.5 per 100m^2, summer flounder 0-7.9 per 100m^2, fourspot flounder 0-3.5 per 100m^2). These congeners appear to co-occur in other estuaries on the eastern seaboard (Weinstein 1979; Burke et al 1991; Burke 1995; Able and Fahay 1998). Juvenile surveys suggest that southern flounder are present throughout the Gulf and East coast of the U.S., but the relative importance of the different paralichthids shifts regionally. In particular, there is a shift to Gulf flounder in the northeastern Gulf and summer flounder on the East coast. While comparisons with other studies indicated that regional trends occur across the range of southern flounder and associated flatfishes, caution must be exercised when interpreting these patterns because experimental design features were quite different among the studies examined (e.g., sampling effort, time and location of collections within the estuary, sampled habitats, gear type used).

Large-scale spatial variation in densities of newly settled southern flounder was found in GBE in 2005, with East Bay appearing to represent a hotspot for settlement. Densities in East Bay were significantly higher than the other two regions of the GBE ranging from 0.4 to 21.7 fish per 100 m^2. Differences in settlement numbers of flatfishes at large scales (e.g. estuarine complex) have been reported and are often linked to spatial variation in environmental conditions, with species often more abundant in areas of the estuary where conditions are within the desired range (Burke et al. 1998). Several studies have determined that salinity is an important environmental scalar for flatfishes particularly during early life (Miller et al. 1991; Gibson 1994). More specifically, Burke

et al. (1991) reported that southern flounder distribution in North Carolina estuaries was determined in part by salinity with highest catches at lower salinities. Therefore, it is possible that higher numbers of southern flounder observed in East Bay were partly a function of salinity since the region had significantly lower salinities (8-17 ppt) than the other bays. While salinity is one possible explanation for observed patterns, other abiotic factors (temperature, dissolved oxygen) varied across the bays sampled and may have influenced observed patterns of habitat use.

In addition to water quality parameters, physical processes (e.g., wind driven currents, tidal forcing) can determine the supply of larvae to a region, and have been shown to influence patterns of settlement as well as nursery habitat use (Bell and Westoby 1986; Jenkins et al. 1997; Brown et al. 2005). Jenkins and Black (1994) compared the temporal settlement patterns of King George whiting (*Sillaginodes punciaia*) to movements of passive particles in a bay system and determined that settlement was largely influenced by coastal hydrodynamic processes. Similarly, Brown et al. (2005) examined the link between physical processes and larval supply to estuarine nurseries in south Texas using a circulation model coupled with a fixed-depth particle transport model, and found that the proximity of the inlet to the nursery ground and the approach path to the inlet were critical factors that influenced larval supply. Although a comparable model does not exist for the GBE, precluding any predictions of transport based on approach path, East Bay sampling sites were closer to the tidal inlet (Rollover Pass) than sites in West Bay or Galveston Bay, possibly increasing the supply of recruits. Also, sites in East Bay are connected to coastal spawning areas by two tidal passes rather than one, which may increase the supply of larvae, particularly if southern flounder

recruits from two different spawning areas converge on the same nursery habitats in East Bay.

Small-scale, habitat-specific variation in density of flatfishes has also been reported and often attributed to a variety of environmental factors (Burke et al. 1991; Miller et al. 1991; Gibson 1994; Guindon and Miller 1995; Burke et al. 1998). Several studies have shown flatfishes to differentiate habitats by bottom sediment or grain size. McConnaughey and Smith (2000) observed that for yellowfin sole (*Limanda aspera*) and Alaskan plaice (*Pleuronectes quadrituberculatus*), sediment texture was the most important factor affecting distribution, more so than temperature or depth. Also, juvenile stone flounder (*Kareius bicoloratus*) and summer flounder have been found to prefer coarser sediments over finer, muddier sediments (Burke et al. 1991; Malloy et al. 1996). In addition to sediment type and grain-size, studies in North Carolina reported a partitioning of habitat by salinity between southern and summer flounder, with southern flounder more abundant in lower-salinity environments (Burke et al 1991). In the present study, no habitat-specific differences in densities were detected, indicating that responses to conditions across nursery habitats were relatively equal. The apparent lack of a habitat effect is not surprising because environmental qualities (DO, temperature, salinity) and substrate condition were similar among habitat types within the same site. Also, the lack of a co-occurring paralichthid (e.g. Gulf flounder or summer flounder) in the study area may have allowed southern flounder to utilize several habitats rather than partitioning available resources to minimize overlap. Making deductions from salinity or sediment type alone is cautioned since these are often linked to one another and temperature.

Peak recruitment of southern flounder juveniles in the GBE occurred in January and February, with the majority of recruits being derived from early-winter (late December to early January) spawning events (based on hatch-date distribution). Findings from the present study were accord with previous reports of early winter spawning of adult southern flounder (Ginsburg 1952; Richards 2006). The timing of peak recruitment of southern flounder in the GBE was slightly earlier than in North Carolina, which occurred in late February (Burke et al. 1991). Observed differences were relatively small and may have been due to natural variability (interannual effect) rather than a regional effect. Nonetheless, it is possible that warmer temperatures in the GBE may have been partly responsible for earlier spawning/settlement events. Collection numbers of southern flounder in the GBE decreased in March and only one southern flounder was captured in April. The decline in numbers was probably a function of emigration from settlement habitat(s) or increasingly successful avoidance of our sampling gear by larger flounder (Rogers et al. 1984; Burke 1995).

The RNA:DNA ratios of the fish in the present study ranged between 6.3 and 15.1, and laboratory-based condition levels (fed versus starved; G.J. Holt, unpublished data) indicated southern flounder in the GBE were in good nutritional condition. Observed ratios are well above a minimum ratio for fed larvae of winter flounder (3.2 to 3.5) and starved Japanese flounder (1.4 to 3.8), and within the ranges found for wild-caught summer flounder (~2.75 to 7.5) (Buckley 1984; Malloy and Targett 1994; Gwak and Tanaka 2001), suggesting food was not a limiting factor in any of the bays or habitats sampled. Rooker and Holt (1997) reported RNA:DNA ratios of wild red drum from Texas estuaries and, similar to the present study, they found the nutritional condition of

wild-caught individuals was well above the minimum or starved baseline estimate. This indicates that prey resources were likely adequate for newly settled southern flounder and food was not a limiting factor for new recruits during the 2005 sampling season.

RNA:DNA ratios of southern flounder in the GBE varied spatially, and ratios were significantly lower in West Bay than in either Galveston Bay or East Bay. Observed differences were possibly related to temperature differences among the bays. Specifically, water temperature in West Bay at the time of collection was approximately 2°C lower than in the other two bays. Moreover, RNA:DNA ratios were highest in the bay with the highest water temperature at time of collection. A positive relationship between water temperature and RNA:DNA ratio is not unexpected as protein synthesis increases with increasing temperature (Fry 1971). In fact, several studies have reported relationships between temperature and RNA:DNA ratio, including work on flatfishes and other marine teleosts (Buckley 1984; Ferron and Leggett 1994; Mathers et al. 1993; Malloy and Targett 1994; Calderone et al. 2003; Mercaldo-Allen et al. 2006). Even though the RNA:DNA ratio appears to vary as a function of temperature, work on larval and metamorphosing Japanese flounder showed no effect (Gwak and Tanaka 2001), suggesting this approach may not be a good proxy for nutritional condition or growth during transition periods. Still, flounder in aforementioned study were much smaller and at different life history stage than those in the present study.

All bays and habitats in the GBE supported equivalent growth, with a mean rate of 0.40 mm d^{-1} (range 0.21-0.76 mm d^{-1}). Our growth estimates were comparable to those for other paralichthids, including summer flounder 0.11-0.27 mm d^{-1} (Necaise et al. 2005), Japanese flounder (*Paralichthys olivaceus*) 0.34-0.93 mm d^{-1} (Gwak et al. 2003),

and fourspot flounder 0.40-0.60 mm d^{-1} (Reichert and van der Veer 1991). Also, observed growth rates for southern flounder fell into the range reported for caged southern flounder in Pamlico Sound, NC: 0.07 to 0.79 mm d^{-1} (Guindon and Miller 1995). Still, field estimates are lower than the 1.2 to 1.4 mm d^{-1} rate obtained in laboratory settings (Peters 1971; Reichert and van der Veer 1991). Differences in growth between lab and field conditions were likely a function of water temperature, which was lower in the field (10-24°C) than controlled conditions in the lab (30°C) (Peters 1971). Moreover, another confounding factor in growth rate comparisons was the size of the fish being compared. Southern flounder in our study were smaller than southern flounder evaluated in other studies (Reichert and van der Veer 1991; Fitzhugh and Rice 1995; Guindon and Miller 1995).

Otolith increment widths are often used to estimate recent growth of fishes within the estuary and several studies have shown the approach to be useful for estimating the condition of flatfishes (Karakiri et al. 1989; Amara and Galois 2004). In the present study, otolith increment widths of southern flounder were used to evaluate their recent growth history. Recent growth was statistically similar among bays even though recent growth estimates were lower (22% on average) in the primary settlement site, East Bay. The East Bay trend was possibly linked to water temperature since it was lower in East Bay over the whole sampling period than in either Galveston Bay or West Bay. Other studies have reported similar relationships between temperature and otolith growth (e.g., Hoff and Fuiman 1993; Barber and Jenkins 2001; Fey 2005). Recent growth was also similar among habitat types sampled. Estimates of recent growth among habitat types have also been reported for post-settlement red drum in other Texas estuaries (Rooker

and Holt 1997; Stunz et al. 2002). Similar to the present study, recent growth rates of red drum did not vary among habitat types, suggesting the growth potential was relatively equal among habitat types. It should be noted that high variability and small sample sizes reduced the power of recent growth assessments on southern flounder and thus could have been responsible for non-significant results. Moreover, recent work by Johnson et al. (2002) indicated that a lag time of 20 to 30 days occurred between start of a stressor (food restriction) and significant changes in otolith growth. Since the majority of southern flounder used for recent growth analysis were new settlers in the GBE (< 15 day post-settlement), it is possible that habitat-specific differences in growth could not be detected due to the reported lag effect. However, thinking is divided on possible lag effects as Folkvord et al. (2000) found that the positive effects of increased prey densities showed up in otoliths within a few days in herring larvae.

SUMMARY AND CONCLUSIONS

Findings from the current study suggest density and condition of newly settled southern flounder vary at the bay scale, suggesting that parts of GBE do not function equally as nurseries. In contrast, at a smaller scale (i.e. habitat-specific), differences in density, condition, and growth were negligible among habitats in close proximity. Physical processes likely play an important role in determining the settlement densities of southern flounder at the GBE scale and evidence from our preliminary sampling in 2004 support this hypothesis (see Appendix 2). Namely, densities were markedly lower and settlement densities were similar among the three bays. Low densities of southern flounder present in the GBE in both years may also indicate that they are recruitment limited. Miller et al. (1991) reported that the main factor(s) controlling year class strength of flatfishes occurs during the pre-settlement period, which lends some support to this idea. Even though settlement numbers in certain areas of the GBE were higher than others (e.g. East Bay), the actual nursery value of these bays will ultimately be related to the contributions made by each bay to adult populations (Beck et al. 2001). While East Bay may indeed be a hotspot for settlement, lower density areas in the GBE may contribute a large portion to the adult population. Across all habitat types examined, we also observed that growth was relatively similar and thus a variety of habitats maintained conditions favorable for growth and survival of newly settled southern flounder. Consequently, it appears that either nursery value of the habitat was similar or newly settled southern flounder are a highly adaptive fish that can utilize a variety of habitat types in the GBE as nursery grounds.

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APPENDIX

Table A-1. Total numbers of collected ichthyofuan from each region of the Galveston Bay Estuary during preliminary sampling round 1 (end of February-beginning of March) in 2004.

Total Ichthyofuna Collected			
Species	**East Bay**	**Galveston Bay**	**West Bay**
Atlantic croaker	540	150	115
Bay whiff	34	25	49
Bighead sea robin	3		1
Clown goby	2	2	8
Code goby			6
Darter goby	112	27	126
Eel species		1	
Fringed flounder		5	
Gulf flounder			1
Gulf killifish	2	3	
Gulf pipefish			6
Highfin goby	1		
Hogchoker		1	
Inland silverside			1
Kingfish		1	
Leptocephalus larvae	4	4	
Naked goby	22	8	1
Non-Paralicthid flatfish		1	
Pinfish	98	87	219
Rainwater killifish			2
Red drum	1	4	
Sailfin molly	1		
Skillet fish		2	
Southern flounder	12	3	15
Southern kingfish		1	
Spot	3	1	1
Texas pipefish			8
Texas silverside		1	
Twoscale goby		1	
Anchovy	1203	1698	66
Blackcheek tonguefish	4	4	2
Mullet spp.	8	31	1
Sheepshead minnow			11

Table A-2. Total numbers of collected flatfish from three regions of the Galveston Bay Estuary during three preliminary sampling rounds in 2004. Round 1=end of February-beginning of March. Round 2=April. Round 3=June.

East Bay			
	Sampling Round		
Species	1	2	3
Bay whiff	34	197	24
Fringed flounder		1	
Gulf flounder			
Hogchoker			
Non-Paralicthid flatfish			
Southern flounder	12	1	
Blackcheek tonguefish	4	6	2

Galveston Bay			
	Sampling Round		
Species	1	2	3
Bay whiff	25	102	1
Fringed flounder	5	1	
Gulf flounder		1	
Hogchoker	1		
Non-Paralicthid flatfish	1		
Southern flounder	3		
Blackcheek tonguefish	4	1	

West Bay			
	Sampling Round		
Species	1	2	3
Bay whiff	49	197	12
Fringed flounder			
Gulf flounder	1	1	
Hogchoker			
Non-Paralicthid flatfish			
Southern flounder	15	2	
Blackcheek tonguefish	2	1	

Table A-3. Complete records of RNA:DNA ratios from sub-sampled newly settled southern flounder collected in the Galveston Bay Estuary in 2005.

SL (mm)	RNA:DNA ratio	RNA per protein	DNA per protein
23.3	13.58	0.1858	0.0263
22.2	10.74	0.0321	0.0029
9.7	6.85	0.0279	0.0038
19.8	11.65	0.0396	0.0031
22	8.06	0.0250	0.0030
23.2	10.08	0.0661	0.0070
19.1	9.57	0.0436	0.0044
12.1	7.79	0.0619	0.0074
12	6.34	0.0307	0.0046
10.4	9.14	0.0505	0.0049
10.6	10.39	0.0603	0.0061
17	11.65	0.0272	0.0023
10.8	6.36	0.0340	0.0053
20.1	8.44	0.0236	0.0026
15.8	10.51	0.0246	0.0023
21.2	11.44	0.0525	0.0040
10	9.49	0.0150	0.0015
17.5	9.60	0.0199	0.0019
11.1	8.35	0.0516	0.0055
22.8	12.27	0.0548	0.0041
25.4	15.19	0.0495	0.0030
10.5	8.10	0.0352	0.0039
21.7	10.57	0.0361	0.0035
11.8	6.99	0.1551	0.0225
19.1	9.81	0.0389	0.0036
14.6	10.21	0.0475	0.0048
17.9	8.24	0.0517	0.0064
22	7.07	0.0386	0.0062
11.7	9.18	0.0436	0.0047
19.3	8.03	0.0332	0.0040
17.1	9.87	0.0325	0.0033
15.6	9.06	0.0332	0.0036
14.9	8.82	0.0369	0.0040
11.9	8.46	0.0680	0.0081
9.7	8.76	0.0517	0.0057
10.7	9.70	0.0562	0.0058
14.2	8.78	0.0292	0.0035
16	8.06	0.0253	0.0030
12.6	8.95	0.0536	0.0064
18.7	9.15	0.0613	0.0067
15.3	9.12	0.0579	0.0060
17.4	9.88	0.0516	0.0052
17.6	9.95	0.0536	0.0056
20.8	9.92	0.0359	0.0033
19.3	8.97	0.0491	0.0049
19.9	9.79	0.0452	0.0041
19.1	8.25	0.0303	0.0035
16	10.69	0.0303	0.0027
18.9	10.68	0.0403	0.0037

VITA

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